10. EQUIPMENT CALIBRATION AND MAINTENANCE

STANDARD 10.1

The Washoe County Sheriff's Office (WCSO) DNA Section uses equipment suitable for the methods employed. All manufacturer's instructions can be found in close proximity to the associated instrumentation in the Biology Unit. All maintenance, calibration, malfunction, and repair documentation will be maintained in the relevant binders in the Biology Unit.

STANDARD 10.2

The WCSO DNA Section's critical equipment will be monitored as listed below:

The following is a list of equipment that will be calibrated every 18 months by the manufacturer or other calibration company and performance checked in-house using the Testo Temperature Verification System every year that it is not sent out for calibration (so that the annual performance check requirement is met).

- Eppendorf Temperature Verification System, multi-probe (Eppendorf)

  If the system stops functioning it will be either taken out of service and sent out immediately for repair to Eppendorf or taken out of service until the next calibration date. If the Eppendorf Temperature Verification System's accuracy falls outside of Eppendorf Service's inaccuracy limits any possible effect on casework will be assessed. The inaccuracy limits are +/- 0.30 ºC for temperatures of 35 ºC and 90 ºC. The DNA Technical Leader or their designee will document their review of the system's calibration by adding the date and their initials or name to the calibration certificate.

The following is a list of equipment that will undergo calibration and maintenance on an annual basis by the manufacturer.

- Testo Temperature Verification System (manufacturer specifications, MCS calibration Inc.)
- Pre-amp and post-amp epMotion 5075 robots pipette calibration (manufacturer specifications, Eppendorf)

The following is a list of equipment that will have a functional assessment and maintenance performed on an annual basis by either the manufacturer or a NIST certified testing device.

- Capillary electrophoresis instrument, model 3130 (manufacturer specifications, Life Technologies)
- 7500 Real-Time PCR instruments (manufacturer specifications, Life Technologies)
- QIAcubes (manufacturer specifications, Qiagen)

- Hamilton easyPunch (manufacturer specifications, Hamilton)

- Pre-amp and post-amp epMotion 5075 robots (manufacturer specifications, pipette calibration, Eppendorf)

- A new calibrated NIST traceable full range thermometer used to perform the monthly check of the ThermoMixers will be purchased annually.

If the equipment is found to be outside of an acceptable range it will be taken out of service until it can be repaired and any possible effect on casework will be assessed if needed. For the 3130 capillary electrophoresis instruments, QIAcubes, 7500 instruments, and epMotion 5075 robots a performance check will be completed prior to their use on casework samples after a preventative maintenance visit or repair. Instruments that require calibration such as the temperature verification systems will be returned to the calibration company so that they can be re-calibrated.

If the Testo Temperature Verification System stops functioning it will be either taken out of service and sent out immediately for repair or taken out of service until the next calibration date. If the Testo Temperature Verification System's accuracy falls outside of MCS Calibration Inc.'s inaccuracy limits any possible effect on casework will be assessed. The inaccuracy limits are +/- 0.3 ºC for temperatures of 0 ºC, 40 ºC, 70 ºC, and 94 ºC. The DNA Technical Leader or their designee will document their review of the system's calibration by adding the date and their initials or name to the calibration certificate.

If an epMotion pipetting tool stops functioning, it will be either taken out of service and sent out immediately for repair to Eppendorf or taken out of service until the next annual calibration. If a pipette's "actual value" falls outside of Eppendorf Service's inaccuracy limits (i.e. "systematic measuring deviation") any possible effect on casework will be assessed. The inaccuracy limits for the TM 50-8 tool are +/- 10% for a volume of 5.0 µL, +/- 2.5% for a volume of 25.0 µL, and +/- 1.2% for a volume of 50.0 µL. The inaccuracy limits for the TS 50 tool are +/- 5% for a volume of 5.0 µL, +/- 1.5% for a volume of 25.0 µL, and +/- 0.8% for a volume of 50.0 µL. The inaccuracy limits for the TS 300 tool are +/- 3% for a volume of 30.0 µL, +/- 1% for a volume of 150.0 µL, and +/- 0.6% for a volume of 300.0 µL. The inaccuracy limits for the TS 1000 tool are +/- 2% for a volume of 100.0 µL, +/- 1% for a volume of 500.0 µL, and +/- 0.6% for a volume of 1000.0 µL. The DNA Technical Leader or their designee will document their review of the calibration by adding the date and their initials or name to each calibration certificate or the calibration summary.

The pipetting tools in use (on deck) on the pre-amp and post-amp epMotion robots will be recorded on a tool log and therefore, do not need to be recorded every time these robots are used by an analyst.

The following is a list of temperature critical instrumentation that will have monthly performance verification. The recordings will be maintained in a binder:

- Refrigerators and Freezers: The Checkpoint monitoring system will be used to continually

monitor the refrigerators and freezers. If the temperature deviates from the acceptable 2 °C to 8 °C range for refrigerators and -15 °C to -25 °C range for freezers for an extended period of time, the monitoring system will send an email alerting the DNA Technical Leader, DNA Supervisor, and the Quality Assurance Manager. The cause of this temperature deviation will be investigated and documented in the Checkpoint program. If the refrigerator/freezer cannot maintain a temperature within the desired range it will be taken out of service and repaired, or replaced with a new refrigerator. Each month a summary of any alerts and corrective actions along with a chart of the temperatures collected for the month will be reviewed, printed out, and placed in a binder.

- ThermoMixers: When the ThermoMixer display shows a temperature of 56 °C, the NIST traceable full range thermometer should fall within 53-59 °C. If the ThermoMixer cannot maintain a temperature within the desired range it will be taken out of service. Until it can be repaired and any possible effect on casework will be assessed if needed.

The following is instrumentation maintenance that will be performed monthly. The recordings will be maintained in a binder:

- 7500 Real-Time PCR instruments: Perform a background check and extraction. Perform a functions test. If the background check fails, i.e. irregular spectral peaks that exceed 72,000 FSU, the block will be cleaned and re-tested. If after repeating the tests the background or functions test continues to fail, Life Technologies will be contacted. The 7500 will be taken out of service until repaired and a performance check completed. Any possible effect on casework will be assessed if needed

- QIAcubes: Perform a tightness test; if the test fails, repeat. If a QIAcube fails the tightness test for a second time, the o-ring in the pipetting system will be replaced and the tightness test will be repeated (DOC ID 6159). If the QIAcube still fails the tightness test, QIAgen will be contacted and the QIAcube taken out of service until it has been repaired and a performance check completed. Any possible effect on casework will be assessed if needed. The o-ring will be visually inspected each month for wear and tear. The o-ring should be changed every six months.

The following is a list of temperature critical instrumentation that will have their temperatures verified quarterly. The recordings will be maintained in a binder:

- Eppendorf Mastercycler Thermal Cyclers: The Eppendorf multi-probe temperature verification system can be used to verify that thermal cycler temperature deviations are less than -2 °C to +1 °C of the set temperature. If temperature deviations are greater than -2 °C to +1 °C of the set temperature, the Eppendorf multi-probe temperature verification system can be used to adjust the Mastercycler's block. This will be followed by a performance check of the adjusted Mastercycler. Any possible effect on casework will be assessed if needed. If the problem persists, the instrument will be taken out of service and Eppendorf's technical support will be contacted. Alternatively, the Testo single probe temperature verification system can be used to verify that thermal cycler temperature deviations are acceptable. If when using the Testo

temperature verification system the temperature deviations are greater than -1.7 °C to +1.3 °C of the set temperature the thermalcycler will be taken out of service until a block adjustment and performance check are completed.

The following is a list of equipment that will be calibrated on a semi-annual basis by an external vendor:

- Manual pipettes (Calibrate, Inc.): If a pipette stops functioning, it will be either taken out of service and sent out immediately for repair to a calibration laboratory or taken out of service until the next semi-annual calibration. If a pipette's "as found" value falls outside of the calibration company's inaccuracy tolerance limits any possible effect on casework will be assessed. The Calibrate Inc. tolerance limits are +/- 16% for a volume of 0.5 µL, +/- 6% for a volume of 2.0 µL, +/- 5% for a volume of 2.5 µL, and +/- 3% for volumes between 5.0 and 1000 µL. The DNA Technical Leader or their designee will document their review of the calibration by adding the date and their initials or name to each calibration certificate or the calibration summary.

  The pipettes used by an analyst for manual DNA extractions are typically the pipettes in that analyst's assigned hood. A record of the hood assigned to each analyst and the pipettes that are present in that hood is saved in the DNA folder on the K drive. If pipettes other than these are used, the name of the pipette(s) will be recorded in the analyst's casework notes, e.g. "PRE3 1000 µL pipette used".

  The pipettes used by an analyst for manual quantitation setup and amplification setup are typically the PRE1 set located at the manual setup area in the pre-amp laboratory. If pipettes other than these are used by an analyst, the name of the pipette(s) will be recorded in the analyst's casework notes, e.g. "Set PRE3".

  The pipettes used to manually add reagents to microtubes for pre-amp epMotion use, e.g. to add 007 positive control, are typically the PRE2 tools located next to the pre-amp epMotion. If pipettes other than these are used by an analyst, the name of the pipette(s) will be recorded in the analyst's casework notes.

  The pipettes used by an analyst during microcon concentration are typically the pipettes in that analyst's assigned hood. If pipettes other than these are used, the name of the pipette(s) will be recorded in the analyst's casework notes

  The pipettes used by an analyst for manual CE setup are typically the POST6 tools located near CE 3130-3 in the post-amp laboratory. If pipettes other than these are used by an analyst, the name of the pipette(s) will be recorded in the analyst's casework notes.

STANDARD 10.3

New and/or repaired instruments are checked for proper function prior to being used on casework or database samples. For example, after a CE 3130 has undergone a major repair involving its optical components, a dilution series of samples must be amplified and run on the CE 3130. Alternatively, if

the repair does not involve the optical portion of the instrument, a plate containing samples that were recently run on a CE 3130 not undergoing a performance check may be re-run on the CE 3130 requiring a performance check. These results can then be compared to the results obtained on the 3130 not undergoing a performance check. A ladder and positive control will also be examined for expected results. After completion of routine annual preventative maintenance, running an amplification positive and negative control along with allelic ladders can suffice as a performance check. Documentation of performance checks will be maintained with the instrument records or service call records. Following calibration and / or repair of the thermal cyclers and annual preventative maintenance and/or repair of the QIAcubes and 7500's, a performance check will be completed. The extent of the performance check will depend on what type of service was performed on the instrument. The Technical Leader will determine the extent of the performance check. All maintenance, calibration, malfunction, and repair documentation will be maintained in the relevant binders in the Biology Unit.